REMARKS

The amendments to the specification correct minor errors. No new matter is believed to be added to the application by this amendment.

Status of the Claims

Claims 1-14 are pending in the application. The amendments to the claims improve their language without reducing their scope.

Objections to The Specification

The Examiner objects to the specification as being unclear. The specification, as amended, is clear and concise.

Rejection under 35 U.S.C. 101

Claim 12 is rejected under 35 U.S.C. 101 as being an improper definition of the process. Applicants traverse.

Claim 12 as amended clearly sets forth a method for the prevention or treatment of a disease. Accordingly, this rejection is overcome and withdrawal thereof is respectfully requested.

Rejection under 35 U.S.C. 112, 2nd paragraph

Claims 1-14 are rejected under 35 U.S.C. 112, 2nd paragraph as being indefinite. Applicants traverse.

The Examiner's comments have been considered, the claims as amended are full, definite and have full antecedent basis. Also, claim 13 sets forth numerical ratios for chromatography solvents. As is known to persons having ordinary skill in the art, these numerical ratios for mixing solvents are on a volume:volume basis. Accordingly, this limitation is clear.

Accordingly, this rejection is overcome and withdrawal thereof is respectfully requested.

Information Disclosure Statement

Applicants thank the Examiner for considering the Information Disclosure Statement filed July 17, 2002 and for making the initial PTO-1449 form of record in the application in the Office Action mailed November 13, 2002.

Conclusion

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Robert E. Goozner (Reg. No. 42,593) at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

Pursuant to 37 C.F.R. §§ 1.17 and 1.136(a), Applicant(s) respectfully petition(s) for a two (2) month extension of time for

filing a reply in connection with the present application, and the required fee of $410.00 is attached hereto.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. §§ 1.16 or 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By _____ #32,380
James M. Slattery, #28,380

JMS/REG/jmb
1624-0132P

P.O. Box 747
Falls Church, VA 22040-0747
(703) 205-8000

Attachment: Substitute Specification and Specification with Hand-written changes

New Gymnemic Acid Derivatives, their Preparation, Pharmaceutical Composition Containing them, and Their Medical use

Field of The Invention

This invention relates to new Gymnemic acid derivatives, their preparation, pharmaceutical composition or extract which contains them, and their medical use, especially the use in the prevention or treatment of the diseases associated with hyperglycemia, hyperlipidemia and platelets aggregation.

Background of The Related Art

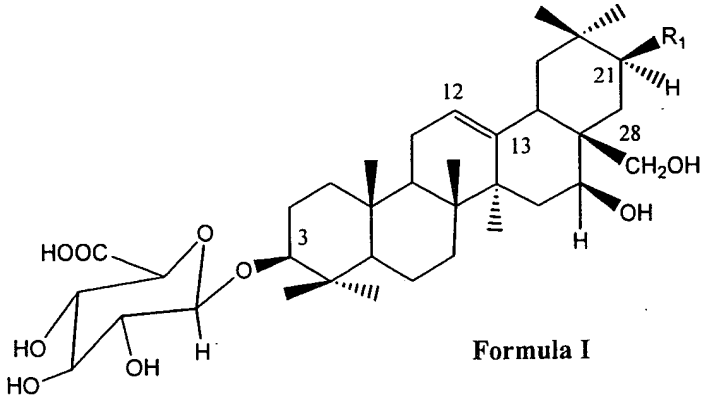
A lot of studies on Gymnemic Acid derivatives have been done and all of these Gymnemic acid derivatives are from the plant called Gymnema cane, which is classified as Gymnema sylvestre. R. Br. In India, it has been used to treat swelling, snake venom toxin, malaria, as a diuretic or to lower blood sugar level. Yet the Gymnemic acid derivatives and their biological activity mentioned in this invention haven't been reported up to this date.

Summary of The Invention

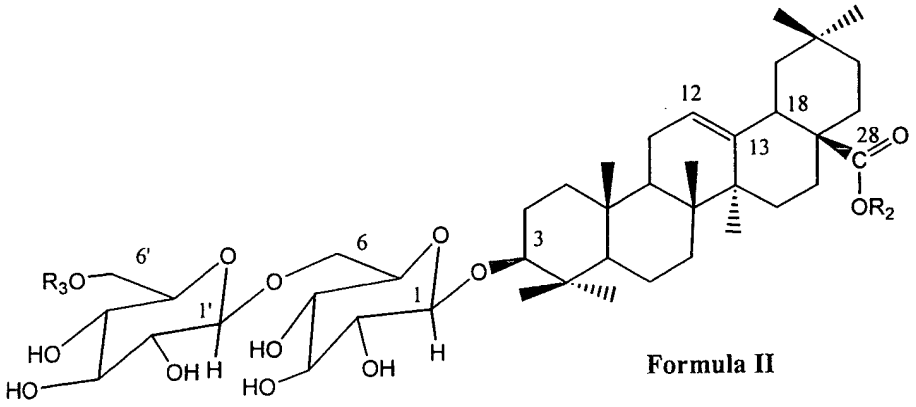
The object of this invention is to find new Gymnemic acid derivatives and develop their medical use.

The inventors have found out new Gymnemic acid derivatives of formula I or II and also their medical use, especially in treating hyperglycemia, hyperlipidemia and platelets aggregation. The invention is now performed based on the discovery mentioned above.

In the first part, this invention concerns Gymnemic Acid derivatives formula I or II,

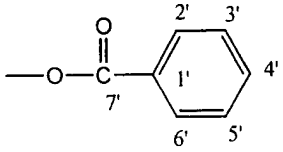


Formula I

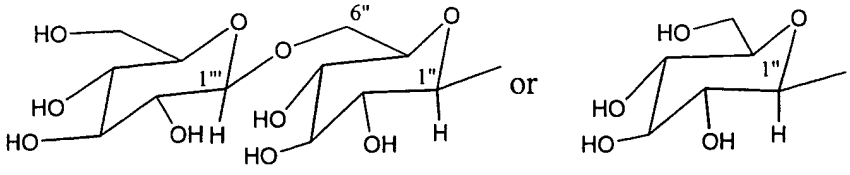


Formula II

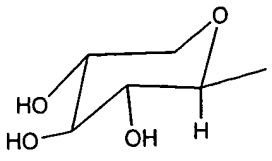
wherein, R_1 is H or the radical represented by the following formula



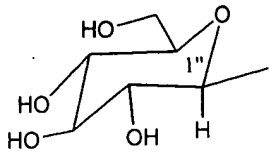
R_3 is H, and R_2 symbolizes the following radical, or



R_3 symbolizes the following radical,



R_2 is H or the following radical,



or pharmaceutically base addition salt thereof.

The second part of this invention relates to pharmaceutical composition which contains at least one kind of Gymnemic Acid derivative of formula I and/or II or pharmaceutical base addition salt thereof as active ingredient, pharmaceutical carrier and excipient.

The third part of the invention involves Gymnemic Acid extract 12.5-40wt% of which is Gymnemic Acid derivative of formula I and/or II.

Another part of this invention relates to pharmaceutical composition for the prevention or treatment of the diseases associated with hyperglycemia, hyperlipidemia and platelets aggregation, which contains at least one kind of Gymnemic Acid derivative of formula I and/or II or pharmaceutical base addition salt thereof as a active ingredient, pharmaceutical carrier and excipient.

Another part of the invention relates to a pharmaceutical composition for

the prevention or treatment of diabetes, which includes at least one kind of Gymnemic Acid derivative of formula I and/or II or pharmaceutical base addition salt thereof as an active ingredient, a pharmaceutical carrier and an excipient.

Another part of this invention relates to pharmaceutical composition for the prevention or treatment of elevated blood lipid level, which contains at least one kind of Gymnemic Acid derivative of formula I and/or II or pharmaceutical base addition salt thereof as a active ingredient, a pharmaceutical carrier and an excipient.

Another part of this invention relates to pharmaceutical composition for the prevention or treatment of platelets aggregation, which contains at least one kind of Gymnemic Acid derivative of formula I and/or II or pharmaceutical base addition salt thereof as a active ingredient, a pharmaceutical carrier and an excipient.

Another part of this invention relates to the preparation of Gymnemic Acid derivative of formula I and II or pharmaceutical base addition salt thereof, which includes the following steps:

a) extracting the plant Gymnema cane with ethanol under reflux and then concentrating;

b) extracting concentrated liquid in step a) with cyclohexane, then extracting with n-butanol, concentrating to dryness under reduced pressure, and then obtaining an ointment;

c) subjecting the ointment in step b) to silica column chromatography with eluent chloroform: methanol=90:10-50:5 or 90:10-60:40, obtaining as eluent Gymnemic acid derivative of formula I and residue;

d) subjecting the residue in step c) to C_{18} column chromatography with

elute as methanol/water (20/80-40/60), obtaining as eluate Gymnemic acid derivative of formula II;

e) if desired, converting the obtained Gymnemic acid derivative of formula I or II into pharmaceutical base addition salt with inorganic or organic base.

Another part of this invention relates to a method of preparation of the extract containing Gymnemic Acid derivative of formula I and II which ranges from 12.5-40wt%, which includes the following steps:

a) extracting Gymnema cane leaves with 60-95% ethanol and concentrating,

b) extracting concentrated liquid in step a) with cyclohexane, then extracting with n-butanol, and then concentrating the extract under reduced pressure.

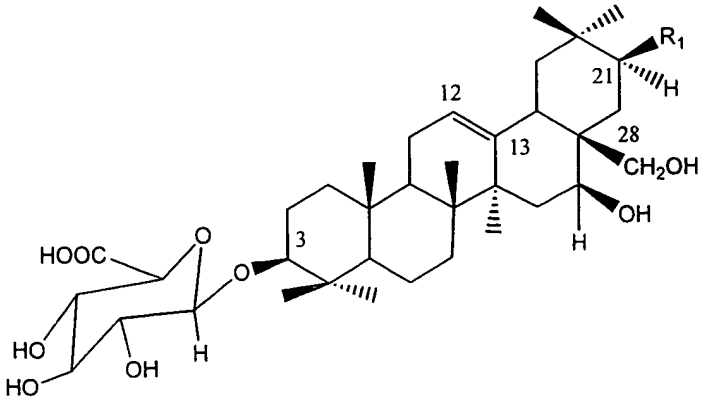
Another aspect of the invention relates to use of Gymnemic Acid derivative of formula I and II or the extract containing Gymnemic Acid derivative of formula I and II for the manufacture of medicament for the prevention or treatment of the diseases and conditions associated with hyperglycemia, hyperlipidemia and platelets aggregation.

Finally, this invention relates to the method of preventing or treating the diseases and conditions associated with hyperglycemia, hyperlipidemia and platelets aggregation, which includes administrating a prophylactic or effective quantity of Gymnemic Acid derivative of formula I and II to a patient suffering from diseases or conditions associated with hyperglycemia, hyperlipidemia and platelets aggregation.

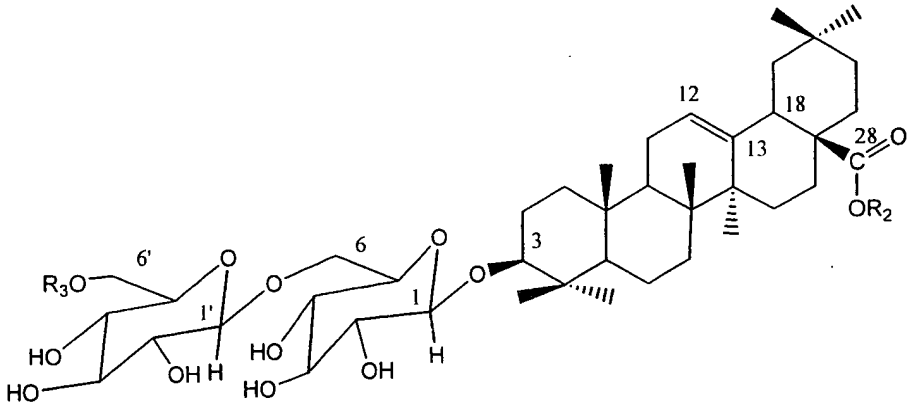
The term "patient" in the invention refers to a mammal, including a human being, and especially a human being.

Detailed Description of the Invention

This invention relates to Gymnemic Acid derivative of formula I and II,

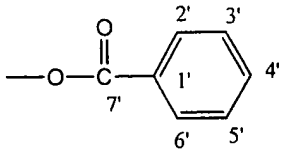


Formula I

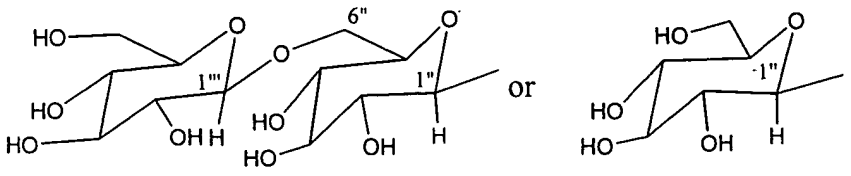


Formula II

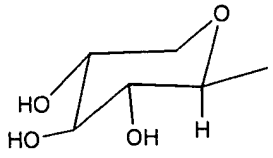
wherein, R_1 is H or the radical represented by the following formula



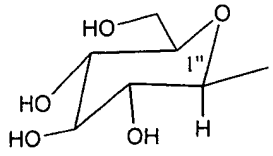
R_3 is H, R_2 is the following group, or



R_3 is the following group,



R_2 is H or the following group,



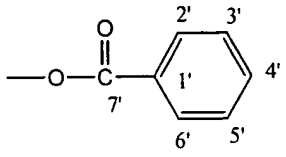
or the pharmaceutical base addition salt.

According to the invention, the pharmaceutical base addition salt of Gymnemic acid of formula I or II includes a salt formed with pharmaceutical inorganic or organic base. The inorganic base, for example, includes alkali or alkali earth metal hydroxide, alkali metal or alkali earth metal carbonate or bicarbonate, alkali metal may be selected from Li, Na, K, alkali earth metal may be selected from Ba, Mg, Ca etc. The organic base, for example, may be triethyl amine etc.

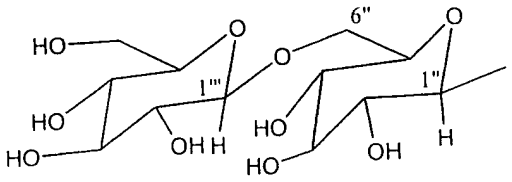
According to this invention, the Gymnemic acid compound preferably is a

Gymnemic Acid compound of formula I wherein R_1 is H.

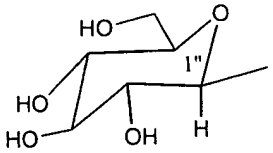
According to the invention, Gymnemic acid compound prefers Gymnemic Acid compound of formula I wherein R_1 is the following radical.



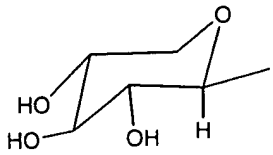
According to the invention, the Gymnemic acid compound is preferably a Gymnemic Acid compound of formula II wherein R_3 is H and R_2 is the following radical.



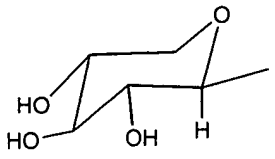
According to the invention, the Gymnemic acid compound is preferably a Gymnemic Acid compound of formula II wherein R_3 is H and R_2 is the following radical.

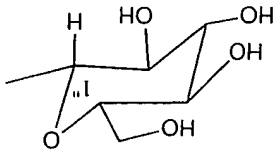


According to the present invention, the Gymnemic acid compound is preferably a Gymnemic Acid compound of formula II wherein R_3 is the following radical and R_2 is H.



According to the invention, the Gymnemic acid compound is preferably Gymnemic Acid compound of formula II wherein both R_3 and R_2 are the following radicals respectively.

R_2 is 

R_3 is 

According to the invention, the pharmaceutical composition contains at least one kind of Gymnemic Acid derivative of formula I and/or II, a pharmaceutical carrier and an excipient. For example, the pharmaceutical composition may include, for example, 1.25-2.10wt% compound A. 0.89-1.50wt% compound B, 2.40-3.80wt% compound C, 2.10-3.40wt% compound D, 2.74-4.60wt% compound E, and 3.24-5.40wt% compound F (compounds A, B, C, D, E and F as defined in examples below.). This pharmaceutical composition can be administrated by gastrointestinal, parenteral or topical administration, such as oral, muscle, subcutaneous, peritonaeum, vein etc. The forms of drug suitable for gastrointestinal administration are for example tablet, capsule, solution, suspension, powder, granulate etc. The forms of drug suitable for parenteral include injection solution, frozen dry powder for injection etc. The drug forms suitable for the topical use are for example, an ointment, cream, paste, patch, and spray. Of all

these forms, oral administration is preferred, and a capsule is the preferred in oral form. The pharmaceutical carrier or excipient of the pharmaceutical composition includes binding agent, filling material, wetting agent, disintegrating agent, surfactant, lubricating agent, diluting agent etc. If desired, a coloring agent, flavoring agent, solubilizer, buffer, etc are also used. The diluting agents in the invention include starch, dextrin, lactose, microcrystallinecellulose, silica gel, etc. Silica gel is preferred. The wetting agents includes water and ethanol, lubricating agents include talcum powder, and magnesium stearate.

The pharmaceutical composition in the present invention can be produced by the known methods in this art. For example, by mixing Gymnemic Acid derivative of formula I and/or II or pharmaceutical base addition salt with pharmaceutical carrier and excipient.

The dose of Gymnemic Acid derivative of formula I and II depends on many factors such as the character and seriousness level of the disease to be prevented or treated, sex, age, weight, individual response, specific compound, administration route and times of administration. Generally the specific dose depends on the judgment of the physician. Generally speaking, the dosage of the pharmaceutical composition Gymnemic Acid derivative of formula I and II can be in the form of single dose and taken 1-4 times per day.

According to this invention, the derivative or pharmaceutical base of the formula I Gymnemic Acid derivative can be prepared as follows:

a) crushing dry leaves of Gymnema cane, then extracting three times with 60-95% ethanol under reflux, two hours for each, combining the extracted liquid and concentrating under reduced pressure until there was no ethanol;

b) extracting the concentrated mixtures in step a) for 3 to 6 times with cyclohexane, then extracting with n-butanol, concentrating to dryness under

reduced pressure, and obtaining dry extract;

c) subjecting the dry extracts in step b) to silica gel column chromatography with an eluent mixture of chloroform and methanol in the ratio of 90:10 to 60:40, and obtaining derivatives of formula I,

d) If desired, converting the derivative of formula I in step c) into a pharmaceutical base salt thereof.

According to this invention, the Gymnemic Acid derivative of formula II can be prepared as follows:

a) Crushing dry leaves of Gymnema cane, then extracting three times with 60-95% ethanol under reflux, two hours for each, combining the extracted liquid and concentrating under reduced pressure until there was no ethanol.

b) extracting the concentrated mixtures for 3 to 6 times with cyclohexane, then extracting with n-butanol, and concentrating to dryness under reduced pressure;

c) mixing the dry extracts in step b) with raw silica gel; separating with thin layer chromatography on silica gel with a mixture of chloroform and methanol at a ratio of 90:10 to 50:50 as eluent, subjecting the residue after elution to C_{18} column chromatography with the eluent being methanol/water (20:80-40:60), and obtaining a derivative of formula II;

d) if desired, converting the derivative of formula II in step c) into the pharmaceutical base salt thereof.

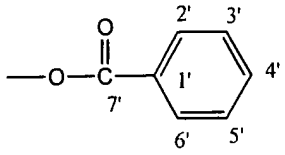
According to this invention, the extract products with 12.5-40 wt% Gymnemic Acid derivative of formula I and formula II can be prepared as follows: raw powder of Gymnema cane leaves were refluxed 1-4 times with 60-95% ethanol, the amount of solvent for each is 6ml/g, and the extraction time is 1-3 hours. The extract mixtures were combined together and distilled under reduced pressure till there was no ethanol, the concentrated mixture was extracted with cyclohexane for 1-3 times, 500ml of solvent was used each time. Then the mixture was extracted for 1-3 times with 500ml n-butanol, all

the extract mixtures were combined and distilled under reduced pressure to obtain the desired product.

This invention gives a further illustration by the preparation examples and biological activity experiment, but it does not infer any limitation to the invention.

Example 1

Preparation of compound A (Gymnemic Acid derivative of formula I wherein the R_1 being H) and compound B (Gymnemic Acid derivative of formula I wherein the R_1 being group as follow)



1000g raw powder of Gymnema cane leaves were refluxed 3 times with 60% ethanol. 6L of solvents were used for each extraction, and the extractions lasted for 2 hours for each time. The extract mixtures were combined together and distilled under reduced pressure until there was no ethanol, the concentrated mixture was extracted with 0.5L cyclohexane and butane for 3 times. All the n-butane extract mixtures were combined and distilled under reduced pressure to obtain 64.0g dry extract product. 32.0g of the dry extract was added into 60g 60-100 mesh rough silica gel, and the mixture was vaporized to dryness on a water pan. 450g 200-300 mesh (m) silica gel was loaded into column by a wet method, then the treated sample was added to be subjected to column separation with elution by 90:10-60:40 mixtures of chloroform-methanol. 80mg of compound A and 60mg of compound B were obtained.

The physical and chemical data of compound A and compound B were

showed as follows:

Compound A:

Amorphous powder: mp 198 - 202 °C; $[\alpha]_{20}^{D}$ +16.0° (c0.10, MeOH); IR ν_{max} 3414 (OH), 1724 (COOH), 1636 (C=C), 1458, 1380, 1054cm^{-1}; ^{1}HNMR (500MHz, pyridin - d5) δ0.86 (3H, s, Me), 0.95 (3H, s, Me), 1.01 (9H, s, 3x Me), 1.32 (3H, s, Me), 1.39 (3H, s, Me), 3.39 (1H, dd, J=4.3 and 11.8Hz, H - 3α), 3.68 (1H, d, J=10.5Hz, H - 28a), 4.43 (1H, d, J=10.5Hz, H - 28b), 4.68 (1H, m, H - 16α), 5.04 (1H, d, J=7.8Hz, H - 1 of gluconic acid), 5.26 (1H, brs, H - 12); ^{13}CNMR (125MHz, pyridin - d5), See table 1 and 2; FAB MS m/z 657 $[M+Na]^{+}$.

Compound B:

Amorphous; mp192 - 195 °C; $[\alpha]_{20}^{D}$ +27.2° (c 0.15, MeOH);

IR ν_{max} 3444 (OH), 1724, 1700, 1635 (C=C), 1457, 1388, 1280, 1074, 720cm^{-1}; ^{1}HNMR (500MHz, pyridin) δ 0.98 (3H, s, Me), 1.01 (3H, s, Me), 1.02 (9H, s, 3 x Me), 1.07 (3H, s, Me), 1.30 (3H, s, Me), 1.34 (3H, s, Me), 1.36 (3H, s, Me), 3.40 (1H, dd, J=4.5 and 12.0Hz, H - 3α), 3.70 (1H, d, J=10.2Hz, H-28a), 4.42 (1H, d, J=10.2Hz, H - 28b), 4.70 (1H, m, H - 16α), 5.10 (1H, d, J=7.8Hz, H- 1 of gluconic acid), 5.70 (1H, dd, J=4.7 and 12.3Hz, H - 21α), 7.47 (3H, overlap, H - 3', - 4' and - 5'), 8.25 (2H, dd, J=1.4 and 4.8Hz, H - 2' and - 6'); ^{13}CNMR (125MHz, pyridin - d5), See table 1 and 2; FAB MS m/z 777 $[M+Na]^{+}$.

Title 1: ^{13}CNMR data of glucoside liquid of compound A and B

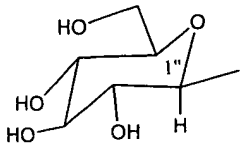
Carbon atom	Compound A	Compound B
1	38.8	38.8
2	26.6	26.6
3	89.0	8 9.0
4	39.5	39.6
5	55.7	55.7
6	18.4	18.4
7	32.9	33.0
8	40.1	40.1
9	47.1	47.1
10	36.7	36.7
11	23.8	23.9
12	122.6	123.1
13	143.9	142.6
14	43.8	43.7
15	36.7	36.8
16	66.6	66.4
17	41.1	43.8
18	44.4	44.2
19	47.1	47.2
20	31.1	36.0
21	34.3	75.6
22	26.2	33.3
23	28.2	28.2
24	16.9	16.9
25	15.7	15.7
26	17.0	17.0
27	27.2	27.0
28	68.9	66.8
29	33.4	29.2
30	24.1	18.8
Acyl 1'		131.6
Acyl 2'		129.9
Acyl 3'		128.9
Acyl 4'		133.2
Acyl 5'		128.9
Acyl 6'		129.9
Acyl 7'		166.3

Table 2: ^{13}CNMR data of saccharide part compound A and B

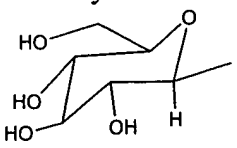
3-position substitution	Compound A	Compound B
Glutamic acid 1	107.3	107.3
Glutamic acid 2	75.6	75.6
Glutamic acid 3	78.2	78.2
Glutamic acid 4	73.5	73.6
Glutamic acid 5	77.8	77.7
Glutamic acid 6	173.1	173.3

Example 2:

Preparation of Compound C (formula II Gymnemic Acid derivative with R_3 as H and R_2 as follow group),

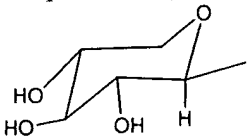


compound D (formula II Gymnemic Acid derivative with R_3 as follows and R_2 as H),

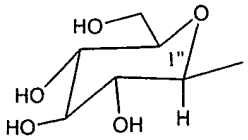


compound E(formula II Gymnemic Acid derivative with R_3 as follow),

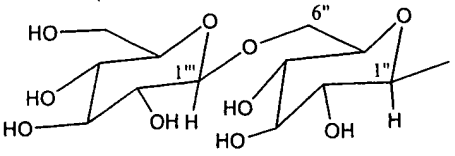
R_2 is



R_3 is



R_2 as follow and compound F(formula II Gymnemic Acid derivative with R_3 as H and R_2 as follow)



1000g raw powder of Gymnema cane leaves were refluxed for 3 times with 75% ethanol. 6.0L solvents were used, 2 hours for each time. The extract mixtures were combined together and distilled under reduced pressure until there was no ethanol, and the condensed mixture was extracted with 0.5L cyclohexane and butane for 3 times. All the n-butane extract mixtures were gathered and distilled under reduced pressure to obtain 72.0g dry extract product. 36.0g dry extract substance was taken and added into 60g 60-100 mesh rough silica gel, and the mixture was vaporized to dryness on a water

pan. 400g 200-400 mu silica for thin-layer separation were loaded into a column in a wet method, then the treated sample was added to undergo column separation with elution by 90:10-50:50 chloroform-methanol mixtures. 130mg compound C, 115mg compound D, 160mg compound E and 195mg compound F were obtained respectively.

The physical and chemical data of compound C were shown as follows:

Amorphous powder; mp206 - 209 °C; $[\alpha]_{20}^{D}$ - 16.0 ° (c 0.11, MeOH); IR ν_{max} 3424 (OH), 1735 (COOR), 1636 (C=C), 1457, 1034cm^{-1}; ^{1}HNMR (400MHz, pyridin - d5) δ 0.82 (3H, s, Me), 0.87 (3H, s, Me), 0.91 (3H, s, Me), 0.97 (3H, s, Me), 1.07 (3H, s, Me), 1.20 (3H, s, Me), 1.23 (3H, s, Me), 3.17 (1H, dd, J=3.5 and 10.2Hz, H - 18), 3.30 (1H, d, J=3.9 and 11.7Hz, H - 3α), 5.37 (1H, brs, H - 12), ^{13}CNMR (100MHz, pyridin - d5), See table 3 and 4; FAB MS m/z 943[M+H]$^{+}$.

The physical and chemical data of compound D were shown as follows:

Amorphous powder; mp 202 - 204 °C ; $[\alpha]_{20}^{D}$ - 3.2° (c 0.15, MeOH); IR ν_{max} 3410 (OH), 1710 (COOR), 1638 (C=C), 1458, 1036cm^{-1}; ^{1}HNMR (400MHz, pyridin - d5) δ 0.87 (3H, s, Me), 0.91 (3H, s, Me), 0.96 (3H, s, Me), 1.02 (3H, s, Me), 1.10 (3H, s, Me), 1.24 (3H, s, Me), 1.29 (3H, s, Me), 3.30 (1H, dd, J=4.5 and 11.5Hz, H - 3α), 5.38 (1H, brs, H - 12), ^{13}CNMR (100MHz, pyridin - d5), See table 3 and 4; FAB MS m/z 935[M+Na]$^{+}$.

The physical and chemical data of compound E were shown as follows:

Amorphous powder; mp212 - 215 °C; [α]20 - 9.6 ° (c 0.20, MeOH); IR ν_{max} 3414 (OH), 1740 (COOR), 1636 (C=C), 1460, 1364, 1044, 896cm^{-1}; ^{1}HNMR (500MHz, pyridin - d5) δ 0.85 (3H, s, Me), 0.90 (3H, s, Me), 0.94 (3H, s, Me), 1.00(3H, s, Me), 3.19 (1H, dd, J=4.0 and 13.7Hz, H - 18), 3.32(1H, d, J=4.4 and 11.7Hz, H - 3 α), 5.40(1H, brs, H - 12), ^{13}CNMR (100MHz, pyridin - d5), See table 3 and 4; FAB MS m/z 943 [M+Na]$^{+}$.

The physical and chemical data of compound F were shown as follows:

Amorphous powder; mp209 - 211 °C ; [α]20 - 12.1° (c 0.12, MeOH); IR ν_{max} 3424 (OH), 1734 (COOR), 1636 (C=C), 1458, 1047cm^{-1}; ^{1}HNMR (400MHz, pyridin - d5) δ 0.87 (3H, s, Me), 0.90 (3H, s, Me), 0.92 (3H, s, Me), 1.00 (3H, s, Me), 1.09 (3H, s, Me), 1.22 (3H, s, Me), 1.26 (3H, s, Me), 3.20 (1H, dd, J=3.5 and 13.6Hz, H - 18), 3.33(1H, d, J=4.4 and 11.5Hz, H - 3 α), 5.39 (1H, brs, H-12), ^{13}CNMR (100MHz, pyridin - d5), See table 3 and 4; FAB MS m/z 1127[M+H]$^{+}$.

Table3: ^{13}CNMR data of glucoside ligand of compound C-F

Carbon atom	Compound C	Compound D	Compound E	Compound F
1	38.8	38.7	38.7	38.7
2	26.6	26.7	26.7	26.7
3	88.9	89.0	89.0	89.0
4	39.4	39.5	39.5	39.5
5	55.7	55.8	55.8	55.8
6	18.4	18.3	18.5	18.5
7	33.0	33.1	33.1	33.1
8	39.8	39.9	39.9	39.9
9	47.9	48.0	48.0	48.0
10	36.9	37.0	37.0	37.0
11	23.7	23.7	23.8	23.7
12	122.9	122.8	123.0	122.9
13	144.0	144.4	144.0	144.1
14	42.0	42.1	42.1	42.1
15	28.2	28.2	28.2	28.2
16	23.3	23.4	23.4	23.4
17	46.9	46.5	47.0	47.0
18	41.6	41.9	41.7	41.7
19	46.2	46.1	46.2	46.3
20	30.7	30.9	30.8	30.8
21	33.9	34.4	34.0	34.0
22	32.5	33.1	32.5	32.5
23	28.1	28.2	28.2	28.3
24	17.0	17.0	17.0	17.0
25	15.5	15.8	15.6	15.6
26	17.4	17.3	17.5	17.5
27	26.0	26.1	26.1	26.1
28	176.4	180.2	176.5	176.5
29	33.1	33.2	33.2	33.2
30	23.6	23.7	23.7	23.7

Table 4: ^{13}CNMR data of saccharic part of compound C-F

3-postion substitution	Compound C	Compound D	Compound E	Compound F
Glc1	106.9	107.0	107.0	106.9
Glc2	75.1	75.0	75.0	75.2
Glc3	78.4	78.3	78.3	78.4
Glc4	71.6	71.5	71.5	71.5
Glc5	77.0	77.0	77.0	77.0
Glc6	70.4	70.4	70.4	70.5
Glc'1	105.4	105.4	105.4	105.4
Glc'2	75.5	75.6	75.6	75.6
Glc'3	78.5	78.5	78.5	78.6
Glc'4	71.7	71.6	71.6	71.7
Glc'5	78.4	76.9	76.9	78.5
Glc'6	62.7	69.8	69.8	62.6
Xyl1		106.0	106.0	
Xyl2		74.9	74.9	
Xyl3		78.0	78.1	
Xyl4		71.1	71.1	
Xyl5		67.0	67.1	
28 position substitution				
Glc"1	95.7		95.8	95.7
Glc"2	74.1		74.1	73.9
Glc"3	78.8		78.9	78.7
Glc"4	71.0		71.1	70.9
Glc"5	79.3		79.3	78.0
Glc"6	62.1		62.2	69.3
Glc'"1				105.3
Glc'"2				75.2
Glc'"3				78.5
Glc'"4				71.7
Glc'"5				78.4
Glc'"6				62.7

Biological activity experiments

Example 1

Effect of compound B on increasing blood sugar in rats caused by

sucrose

Female SD rats fasted for 24 hours and were randomly divided into several groups. Test groups are given 50, 100, 200mg/kg compound B, and the positive-control group was given 100mg/kg phenformin. The normal group, control group and blank group were given the same amount of water. The given medicine volume was 10mg/kg, after 30 minutes each group was given saccharose 1/kg(5ml/kg) except the normal group, and blood was extracted from the eyes of rats after 30, 60 and 120 minutes respectively, the content of glucose in the serum was measured.

The result was, after the rats were given saccharose for 30, 60 minutes, the value of blood sugar increased apparently. The compound B 200mg/kg and phenformin 100mg/kg within 30 minutes can both reduce the increased value of blood sugar remarkably, and the strength of the two compounds was similar. See table 5 for the results.

Table 5: The effect of compound B on the increasing blood sugar in rats caused by sucrose

(X±SD, n=10)

group	dose (mg/kg)	Value of blood sugar (mmol/L)		
30minutes	60 minutes	120 minutes		
Normal group		3.56±0.64	4.12±0.72	3.76±0.69
control group		6.58±0.87$^{\Delta\Delta}$	5.93±1.27$^{\Delta\Delta}$	4.54±1.37
compound B	50	6.03±0.86	6.42±0.78	4.26±1.03
	100	5.12±1.29**	5.77±1.09	4.53±0.94
	200	4.43±0.72**	4.73±0.83**	4.07±0.70
phenformin	100	4.24±0.87**	4.74±0.90*	4.79±1.03

$^{\Delta\Delta}$P<0.01, compared with normal group; *P<0.05, *P<0.01, compared with control group.

Example 2

The effect of compound B on the contents of TG, cholesterol in the serum of hyperlipidemia rats

Male SD rats with a weight of 130-170g, normal group, was given common food. Other groups were given food having high lipid content (1%cholesterol, 10%lard, 0.3% cholic acid, 0.2% methylthio imidazole and 88.5% common forage, made into a block.). For 14 sequential days, rats fasted for 12 hours were measured by reagent box method to obtain the contents of TG and cholesterol in serum. Then they were divided according to the value of blood fat content into different group. The experimental group was given 50, 100. 200mg/kg compound B, the positive-control group was given clofibrate 100mg/kg, and the control group was given water. The given medicine volume was 10ml/kg, for 10 days, each group was still given high fat forage for 5 days before being given medicine, and common forage was given in the later 5 days. The rats were fasted for 11 hours before being given the final administration and blood of each rat was extracted to obtain the content of TG and cholesterol in serum 1 hour after being given medicine.

The results show that 10 days after rats were given forage having high grease, the contents of TG and cholesterol increased. Compound B 50, 100, 200mg/kg and clofibrate 100mg/kg can both reduced the contents of TG and cholesterol in blood serum of hyperlipidemia rats, and compound B 200mg/kg has the same effect as 100mg/kg clofibrate in reducing hyperlipidemia, see table 6.

Table 6: The effect of compound B on the contents of blood lipid in hyperlipidemia rats

(X±SD, n=9 - 10)

group	dose (mg/kg)	TG (mmol/L)		Total cholesterol (mmol/L)	
		Before administration	After administration	Before administration	After administration
Normal group		1.02±0.22	1.04±0.15	2.43±0.41	1.99±0.47
control group		2.64±0.82	3.04±0.93	4.10±0.51	$4.77\pm0.63^{\Delta\Delta}$
compound B	50	2.72±0.61	2.41±0.44	4.29±0.60	3.92±0.58**
	100	2.54±0.90	1.75±0.53**	4.02±0.59	2.94±0.66**
	200	2.72±0.76	1.37±0.40**	4.18±0.61	2.31±0.74**
clofibrate	100	2.51±0.77	2.72±0.74	4.33±0.51	2.15±0.76**

$^{\Delta\Delta}P<0.01$, compared with normal group; **$P<0.01$, compared with control group

Example 3

Effect of compound B on blood platelet aggregation in rabbits in vitro.

Blood was taken from rabbit heart by puncture, to which was added 3.8 % potassium citrate for anticoagulation (1:9). Centrifugation for 15 minute at 1000rpm takes the upper layer as rich blood platelet plasma (prp), and then centrifugation for 10 minutes with 4000 rpm takes the supernatant as poor blood platelet plasma (ppp). Transfer ppp (200ul) to a nephelotube, and add into different concentrations of physiological brine solution 10ul of the compound B. The final concentrations are respectively 250, 500, 1000 μg/ml. 10 μl physiological brine of aspirin was added to a positive control tube, then it was put into a measuring cell after warming for 2 minutes at 37°C. 10ul of physiological brine solution of ADP sodium salt was added with stirring. The final concentration is $1.0x10^{5}$M. The maximal aggregation ratio on PAM-1 type of blood platelet instrument was observed within 3 minutes.

The result shows that the compound B 500, 1000 μg/ml and aspirin 250μg/ml obviously inhibit blood platelet from aggregating.

Table 7: Effect of compound B on blood platelet aggregation in rabbit (X± SD, n=8)

Group	Final concentration	Maximal aggregation ratio (%)	Inhibition ratio
Control group		47.9±5.2	
compound B	250	43.6±7.0	9.0
	500	35.9±4.5**	25.1
	1000	27.8±4.8**	42.0
Aspirin	250	23.7±6.0**	50.3

**P<0.01, compared with control group

Example 4

Effect of compound F on blood sugar elevation in rat.

Male Kun Ming strain mice are divided into randomly experimental groups, and they respectively took orally the compound F at 50, 100, 2000mg/kg. The positive control group took orally glybenclamide 50mg/kg, and the blank control group and normal control group take orally the same distilled water. The volume of medicine given is 20ml/kg, lasting 7 days. They are forbidden to give feedstuff 10 hours before the last time of administration. Each group is given 2.5g/kg (10ml/kg) dextrose solution except of normal control group. Before and after 30 minute of administration of dextrose, 100ul of blood was sampled from the eyepit, and the content of dextrose in serum was measured by way of dextrose oxygenation enzyme.

Result

30 minutes after mice orally took dextrose, the blood sugar obviously rises. Both the compound F 100, 200mg/kg and 50mg/kg inhibits blood sugar in mice from rising. The function of the compound B 200mg/kg and glybenclamide 500mg/kg in lowering blood sugar is similar, which may be seen in table 8.

Table 8

Group		Dose (mg/kg)	Value of blood sugar
		0 minute	30 minutes
Normal Group		6.20±1.01	6.64±1.04
Control Group		6.55±1.16	13.94±3.22
compound F	50	6.79±1.16	12.01±1.88
	100	6.09±1.34	9.59±2.25**
	200	6.42±0.99	9.16±1.08**
glybenclamide	50	4.48±0.83**	8.18±1.72**

P<0.01,compared with normal group; **p<0.01,compared with control group

Example 5

Effect of compound F on the content of triglycerides and cholesterol in the serum of hyperlipidemia rat.

Male SD rats weighing 130-170g were used. The normal group was given general feedstuff, and the other groups were given high-fat (1% cholesterol, 10% pig oil, 0.3% cholic acid, 0.2 % methylthio imidazole and 88.5 % normal feedstuff are made stuff by oneself). After the feedstuff is run for 14 days and the rats were forbidden to eat for 12 hours, the content of triglycerides and cholesterol in rat's serum was measured. Then, the rats were grouped randomly according to blood lipid value. The experiment group was given compound F (50, 100, 200mg/kg.), the positive-control group was orally given clofibrate (100mg/kg), and the control group was given distilled water. The volume of administration was 10ml/kg, lasting 10 days. Each group was given high-fat feedstuff in the first 5 days of giving

drugs, and then general feedstuff in the next 5 days. Fasting of 11 hours is conducted before the last time of giving drugs. After giving drugs for 1 hour, blood was taken and the content of ester and cholesterol in the blood serum was measured.

Result

The content of TG and cholesterol in the blood serum of rat elevates obviously after given high-fat feedstuff for 10 days. 50mg/kg, 100mg/kg, 200mg/kg of compound F and 200mg/kg clofibrate make the level of triglycerides and cholesterol in blood serum of rat with high-fat blood diseases lower. The action 200mg/kg of the compound F is the similar as to that of 100mg/kg of clofibrate in the function of lowering blood fat. (table 9)

Table 9: The effect of compound F on the content of blood fat of rat with high-fat blood disease. (X±SD, n=9-10)

Group	dose (mg/kg)	triglycerides (mmol/L)		Total cholesterin (mmol/L)	
		Before administration	After administration	Before administration	After administration
Normal group		1.02±0.22	1.04±0.15	2.43±0.41	1.99±0.47
Control group		2.64±0.82	3.04±0.93	4.10±0.51	4.77±0.63
compound B	50	2.72±0.61	2.41±0.44	4.29±0.60	3.92±0.58**
	100	2.54±0.90	1.75±0.53**	4.02±0.59	2.94±0.66**
	200	2.72±0.76	1.37±0.40**	4.18±0.61	2.31±0.74**
Clofibrate	100	2.51±0.77	2.72±0.74	4.33±0.51	2.15±0.76**

$^{\Delta\Delta}P<0.01$, (compared with normalgroup); **P<0.01(compared with control group)

Example 6. Effect of compound F on blood platelet aggregation in rabbit.

Take blood from rabbit heart by puncturing, add 3.8% of potassium citrate for anticoagulation (1:9), centrifuge for 15 minutes at 1000rpm, take

the upper layer as blood platelet rich plasma (prp), and then centrifuge for 10 minutes at 4000rpm, and take supernatant as blood platelet poor plasma (ppp). The final concentration of compound F is respectively 250, 500, 1000 μg/ml, and the final concentration is respectively 250, 500, 1000 ug/ml. Add 10ul of physical brine of aspirin to the positive-control tube to a final concentration of 250 μg/ml, and add 10μl of physical brine to the control tube to a final concentration of 250 μg/ml. Observe the maximal aggregation ratio on PAM-1 type instrument of blood platelet aggregation within 3 minute.

The result shows that 500, 1000 μg/ml of the compound F and aspirin 250 μg/ml obviously inhibit the aggregation of blood platelet.

Table 10: The effect of the compound F on aggregation of rabbit's blood platelets in vitro.

Group	Final concentration (μg/ml)	Maximal aggregation rate (%)	Inhibition rate (%)
Control		47.9±5.2	
Compound F	250	43.6±7.0	9.0
	500	35.9±4.5**	25.1
	1000	27.8±4.8**	42.0
Aspirin	250	23.7±6.0**	50.3

**P<0.01(compared with the control)

Example 7

Effect of compound B on blood sugar in normal mice.

Male Kun Ming strain mice are divided into random experimental groups, and they respectively take orally the compound B at 50, 100, 2000mg/kg. The positive control group orally took tolbutol at 100mg/kg. The blank control group took orally same distilled water. The volume of medicine given is 20ml/kg, lasting 14 days. The test drug was administered (provided that they are pre-forbidden to give food 5 hrs before administration) after the days 1, 3, 7, 14 of administration. 3hrs after administration, blood (10ul) was

taken from the eyepit. The content of dextrose in serum was measured by reagent box.

Result

Compound B 50, 100, 200mg/kg by continuous administration for 14 days has no obvious effect on blood sugar of normal mice, but tolbutol starting from day 3 of administration show obvious effect for lowering the blood sugar of normal mice. The result is also seen in table 11.

Table 11. Effect of compound B on blood sugar in normal mice.

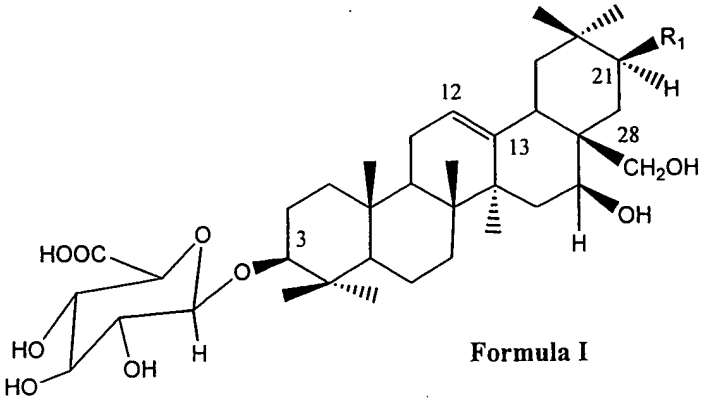
(X±SD, n=10)

Group	Dose (mg/kg)	Value of blood sugar			
		1	3	7	14 (day)
control Group		5.21±1.10	7.10±1.30	8.56±0.74	7.52±1.29
compound B	50	5.84±0.94	7.56±0.92	8.51±1.06	8.27±0.66
	100	6.48±1.28	7.73±2.26	8.71±0.97	7.45±1.59
	200	6.41±1.04	6.28±1.19	8.46±0.88	7.86±1,56
tolbutol	100	6.48±1.18	5.22±0.80**	6.62±0.96	5.75±1.02**

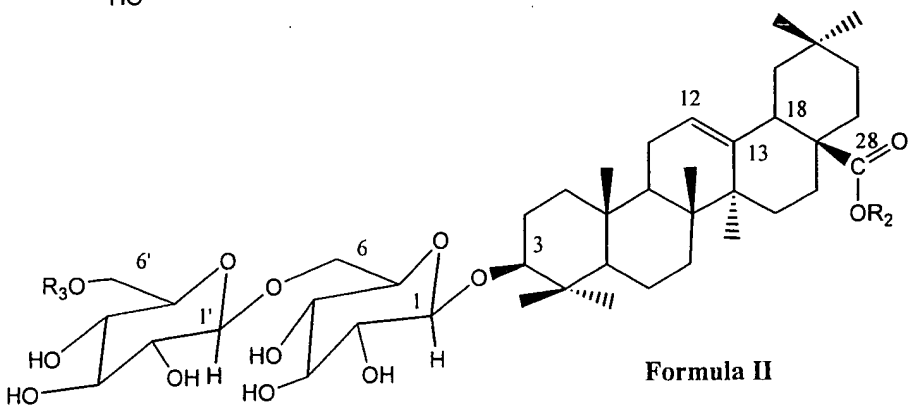
**$p<0.05$, compared with control group

What is claimed is

1. Gymnemic Acid derivative of general formula I or general formula II,

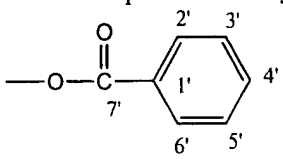


Formula I

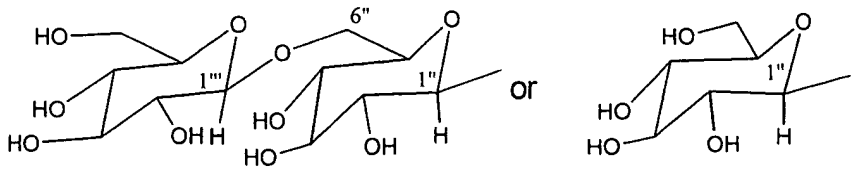


Formula II

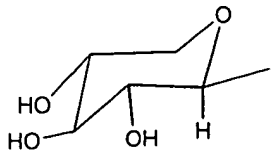
wherein, R_1 is H or the radical represented by the following formula



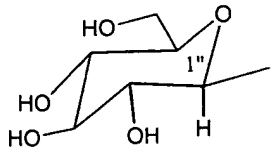
R_3 is H, and R_2 symbolizes the following radical, or



R_3 symbolizes the following radical,



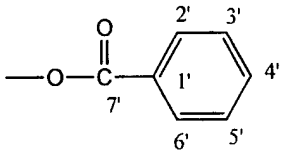
R_2 is H or the following radical,



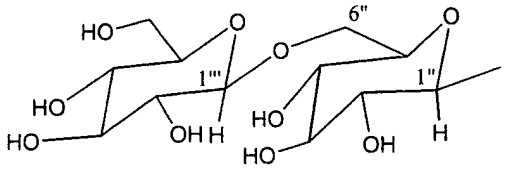
or pharmaceutically base addition salt thereof.

2. Gymnemic Acid derivatives of claim 1,wherein R_1 in formula I is hydrogen.

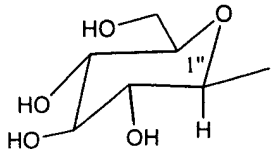
3. Gymnemic Acid derivatives of claim 1,wherein R_1 in formula I is a group of the formula:



4. Gymnemic Acid derivatives of claim 1, wherein R_3 in formula II is hydrogen, R_2 is group of formula:

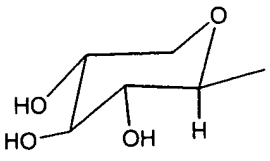


5. Gymnemic Acid derivatives of claim 1, wherein R_3 in formula II is hydrogen, R_2 is group of formula:

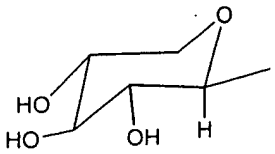


6. Gymnemic Acid derivatives of claim 1, wherein R_2 in formula II is

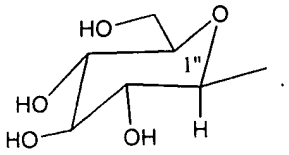
hydrogen, R_3 is group of formula:



7. Gymnemic Acid derivatives of claim 1, wherein R_3 in formula II is group of formula



R_2 is group of formula:



8. Pharmaceutical composition which contains at least one kind of Gymnemic Acid derivative of formula I and/or II or pharmaceutical base addition salt thereof as active ingredient, pharmaceutical carrier and excipient.

9. Pharmaceutical composition for the prevention or treatment of the diseases associated with hyperglycemia, hyperlipidemia and platelets aggregation, which contains at least one kind of Gymnemic Acid derivative of formula I and/or II or pharmaceutical base addition salt thereof as a active ingredient, pharmaceutical carrier and excipient.

10. A composition of claims 1 or 2,which contains Gymnemic Acid derivative of formula I and/or II ,wherein based on the weight of the composition, the content of compounds A, B, C, D, E and is 1.25-2.10% compound A,

0.89-1.50% compound B, 2.40-3.80% compound C, 2.10-3.40% compound D, 2.74-4.60% compound E and 3.24-5.40% compound F.

11. An extract of Gymnema sylevestre.R.Br which contains 12.5-40wt% Gymnemic acid derivatives of formula I and formula II.

12. Use of Gymnemic Acid derivative of formula I and II or the extract containing Gymnemic Acid derivative of formula I and II for the manufacture of medicament for the prevention or treatment of the diseases and conditions associated with hyperglycemia, hyperlipidemia and platelets aggregation.

13. A method of the preparation of Gymnemic Acid derivative of formula I and II or pharmaceutical base addition salt thereof, which includes the following steps:

a) extracting the plant Gymnema cane with ethanol under reflux and then concentrating;

b) extracting concentrated liquid in step a) with cyclohexane , then extracting with n-butanol, concentrating to dryness under reduced pressure, and then obtaining a ointment;

c) subjecting the ointment in step b) to silica column chromatography with elute as chloroform: methanol=90:10-50:5 or 90:10-60:40, obtaining Gymnemic acid derivative of formula I and residue;

d) subjecting the residue in step c) to C_{18} column chromatography with elute as methanol/water (20/80-40/60), obtaining Gymnemic acid derivative of formula II;

e) if desired, converting the obtained Gymnemic acid derivative of formula I or II into pharmaceutical base addition salt with inorganic or organic base.

Abstract

This invention relates to new Gymnemic acid derivatives, their preparation, pharmaceutical composition or extract which contains them, and their medical use, especially the use in the prevention or treatment of the diseases associated with hyperglycemia, hyperlipidemia and platelets aggregation.

New Gymnemic Acid Derivatives, their Preparation, Pharmaceutical Composition Containing them and Their Medical Use

The Invention Field

This invention relates to new Gymnemic acid derivatives, their preparation, pharmaceutical composition or extract which contains them, and their medical use, especially the use in the prevention or treatment of the diseases associated with hyperglycemia, hyperlipidemia and platelets aggregation.

Background of technology

A lot of study on Gymnemic Acid derivatives have been done and all of these Gymnemic acid derivatives are from the plant called Gymnema cane, which is classified as Gymnema sylvestre. R. Br. And in India, it has been used to treat swelling, snake venom toxin, malaria, as a diuretic or to lower blood sugar level. Yet the Gymnemic acid derivatives and their biological activity mentioned in this invention haven't been reported up to date.

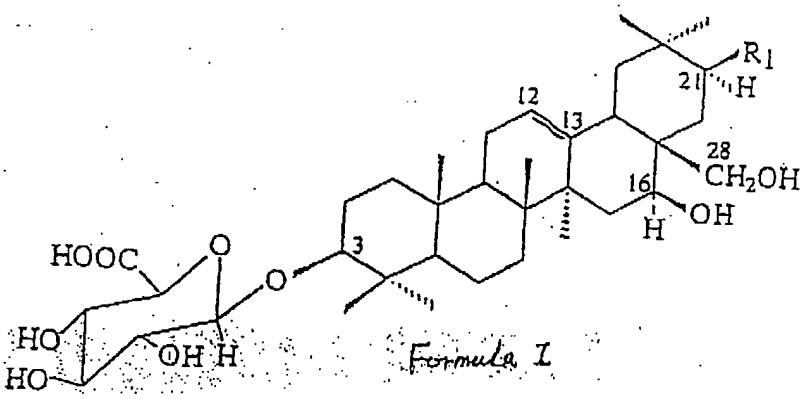
The object of this invention

The object of this invention is to find new Gymnemic acid derivatives and develop their medical use.

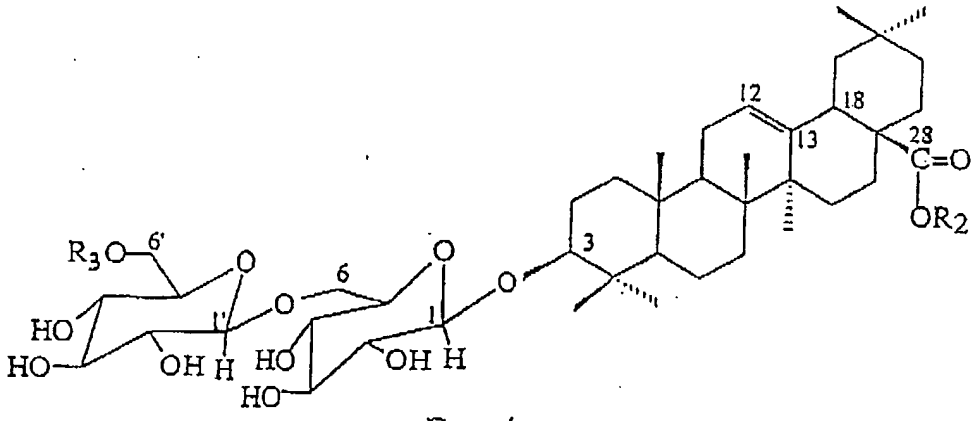
Description of this invention

This inventor has found out new Gymnemic acid derivatives formula I or II and further their medical use, especially in treating hyperglycemia, hyperlipidemia and platelets aggregation. The invention is now performed based on the discovery mentioned above.

In the first part, this invention concerns Gymnemic Acid derivatives formula I or II,

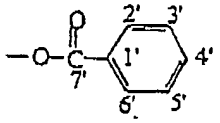


Formula I

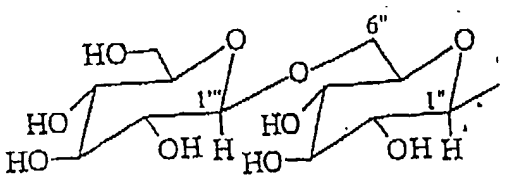


Formula II

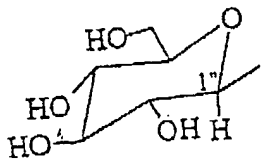
wherein, R_1 is H or the radical represented by the following formula



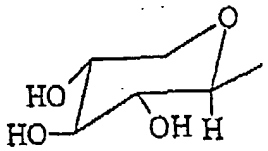
R_3 is H, and R_2 symbolizes the following radical, or



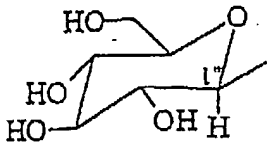
or



R_3 symbolizes the following radical,



R_2 is H or the following radical ,



or pharmaceutically base addition salt thereof.

The second part of this invention relates to pharmaceutical composition which contains at least one kind of Gymnemic Acid derivative of formula I and/or II or pharmaceutical base addition salt thereof as active ingredient, pharmaceutical carrier and excipient.

The third part of the invention involves Gymnemic Acid extract, 12.5—40wt% of which is Gymnemic Acid derivative of formula I and/or II.

Another part of this invention relates to pharmaceutical composition for the prevention or treatment of the diseases associated with hyperglycemia, hyperlipidemia and platelets aggregation, which contains at least one kind of Gymnemic Acid derivative of formula I and/or II or pharmaceutical base addition salt thereof as a active ingredient, pharmaceutical carrier and excipient.

Another part of the invention relates to a pharmaceutical composition for

the prevention or treatment of diabetic, which includes at least one kind of Gymnemic Acid derivative of formula I and/or II or pharmaceutical base addition salt thereof as active ingredient, officinal carrier and excipient.

Another part of this invention relates to pharmaceutical composition for the prevention or treatment of higher blood lipid level, which contains at least one kind of Gymnemic Acid derivative of formula I and/or II or pharmaceutical base addition salt thereof as a active ingredient, pharmaceutical carrier and excipient.

Another part of this invention relates to pharmaceutical composition for the prevention or treatment of platelets aggregation, which contains at least one kind of Gymnemic Acid derivative of formula I and/or II or pharmaceutical base addition salt thereof as a active ingredient, pharmaceutical carrier and excipient.

Another part of this invention relates to the preparation of Gymnemic Acid derivative of formula I and II or pharmaceutical base addition salt thereof, which includes the following steps:

a) extracting the plant Gymnema cane with ethanol under reflux and then concentrating;

b) extracting concentrated liquid in step a) with cyclohexane, then extracting with n-butanol, concentrating to dryness under reduced pressure, and then obtaining a ointment;

c) subjecting the ointment in step b) to silica column chromatography with elute as chloroform: methanol=90:10—50:5 or 90:10—60:40, obtaining Gymnemic acid derivative of formula I and residue;

d) subjecting the residue in step c) to C_{18} column chromatography with elute as methal/water (20/80-40/60), obtaining Gymnemic acid derivative of formula

II;

e) if desired, converting the obtained Gymnemic acid derivative of formula I or II into pharmaceutical base addition salt with inorganic or organic base.

Another part of this invention relates to a method of preparation of the extract containing Gymnemic Acid derivative of formula I and II which ranges from 12.5-40wt%, which includes the following steps:

a)extracting Gymnema cane leaves with 60-95% ethanol and concentrating,

b)extracting concentrated liquid in step a) with cyclohexane, then extracting with n-butanol, and then concentrating the extract under reduced pressure.

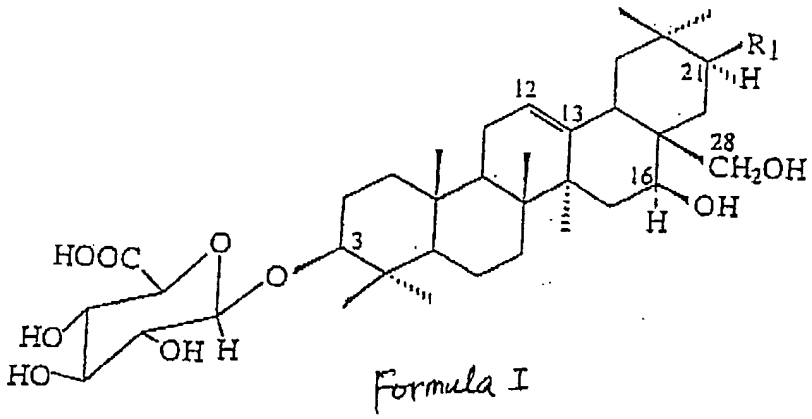
Another aspect of the invention relates to use of Gymnemic Acid derivative of formula I and II or the extract containing Gymnemic Acid derivative of formula I and II for the manufacture of medicament for the prevention or treatment of the diseases and conditions associated with hyperglycemia, hyperlipidemia and platelets aggregation.

Finally, this invention relates to the method of preventing or treating the diseases and conditions associated with hyperglycemia, hyperlipidemia and platelets aggregation, which includes administrating prophylactic or treatment effective quantity of Gymnemic Acid derivative of formula I and II to the patient suffered from diseases or conditions associated with hyperglycemia, hyperlipidemia and platelets aggregation.

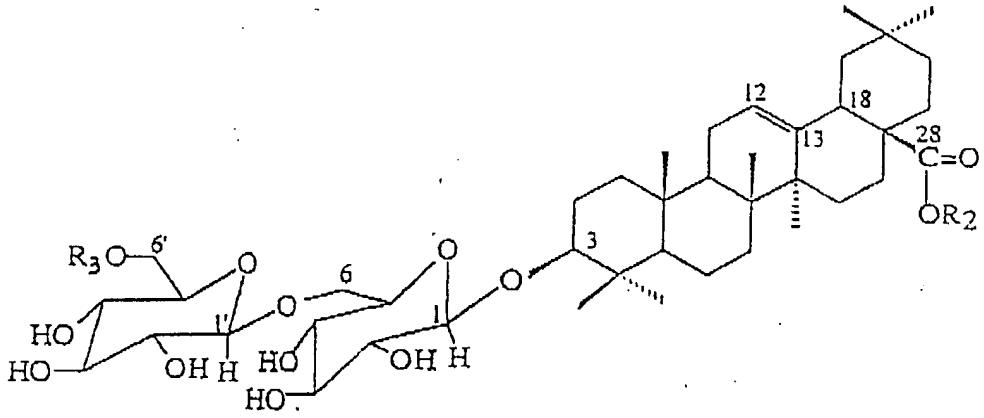
The term "patient" in the invention refers to mammal, including human being, and especially human being.

Detailed Description of the Invention

This invention relates to Gymnemic Acid derivative of formula I and II,

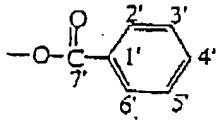


Formula I

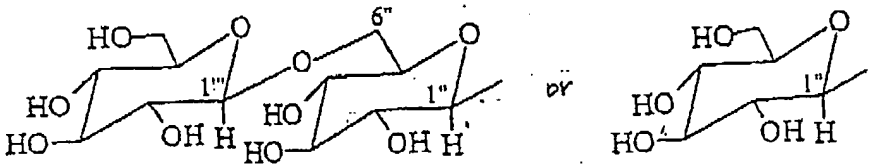


Formula II

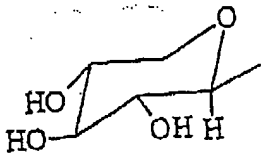
Wherein, R_1 is H or the group of the following formula



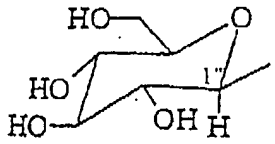
R_3 is H, R_2 is the following group, or



R_3 is the following group,



R_2 is H or the following group,

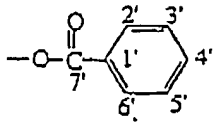


or pharmaceutical base addition salt.

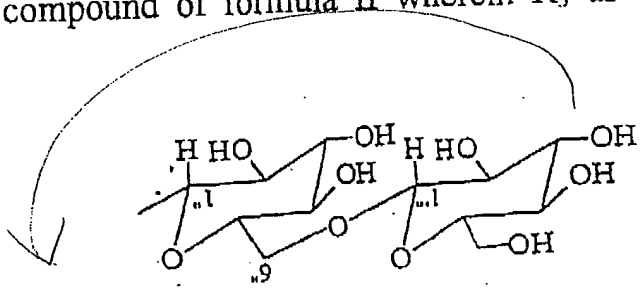
According to the present invention, pharmaceutical base addition salt of Gymnemic acid of formula I or II includes a salt formed with pharmaceutical inorganic or organic base, inorganic base, for example, includes alkali or alkali earth metal hydroxide, alkali metal or alkali earth metal carbonate or bicarbonate, alkali metal may be selected from Li、Na、K, alkali earth metal may be selected from Ba, Mg, Ca etc. The organic base, for example, may be triethyl amine etc.

According to this invention, Gymnemic acid compound prefers Gymnemic Acid compound of formula I wherein R_1 is H.

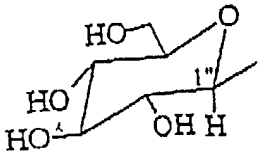
According to the present invention, Gymnemic acid compound prefers Gymnemic Acid compound of formula I wherein R_1 as the following radical.



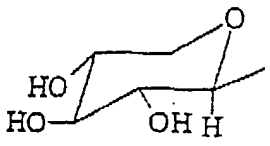
According to the present invention, Gymnemic acid compound prefers Gymnemic Acid compound of formula II wherein R_3 as H and R_2 as the following radical.



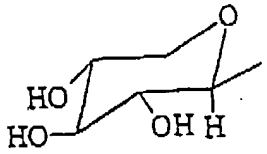
According to the present invention, Gymnemic acid compound prefers Gymnemic Acid compound of formula II wherein R_3 as H and R_2 as the following radical.

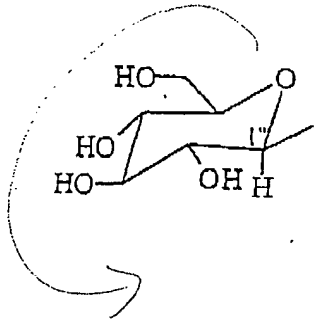


According to the present invention, Gymnemic acid compound prefers Gymnemic Acid compound of formula II wherein R_3 as the following radical and R_2 as H.



According to the present invention, Gymnemic acid compound prefers Gymnemic Acid compound of formula II wherein both R_3 and R_2 as the following radical respectively.

R_2 is 

R_3 is 

According to this invention, the pharmaceutical composition mentioned here contains at least one kind of Gymnemic Acid derivative of formula I and/or II, and pharmaceutical carrier and excipient. For example, the pharmaceutical composition may include, for example, 1.25-2.10wt% compound A, 0.89-1.50wt% compound B, 2.40-3.80wt% compound C, 2.10-3.40wt% compound D, 2.74-4.60wt% compound E, and 3.24-5.40wt% compound F (compounds A, B, C, D, E and F as defined in examples below.). This pharmaceutical composition can be administrated by gastro intestine parenteral or topical administration, such as oral, muscle, subcutaneous, peritonaeum, vein etc. The forms of drug suitable for intestine administration are for example tablet, capsule, solution, suspension, powder, granulate etc. The forms of drug suitable for parenteral include injection solution, frozen dry powder for injection etc. The drug forms suitable for the topical are for example, ointment, cream, paste, patch, and spray. Of all these forms, oral administration is preferred while capsule is preferred in oral form. The

pharmaceutical carrier or excipient of the pharmaceutical composition includes binding agent, filling material, wetting agent, disintegrating agent, surfactant, lubricating agent, diluting agent etc. If desired, coloring agent, flavoring agent, solubilizer, buffer, etc, are also used. The diluting agents in the invention include starch, dextrin, lactose, microcellulose, silica gel, etc. And silica gel is preferred. The wetting agents includes water and ethanol, lubricating agents include talcum powder, stearic magnesium and magnesium stearate.

The pharmaceutical composition in the present invention can be produced by the known methods in this art. For example, mixing Gymnemic Acid derivative of formula I and/or II or pharmaceutical base addition salt with pharmaceutical carrier and excipient.

The dose of Gymnemic Acid derivative of formula I and II depends on many factors such as the characters and seriousness level of the disease to be prevented or treated, sex, age, weight, individual response, specific compound, administration route and times of administration. Generally the specific dose depends on the judgment of the physician. Generally speaking, the dosage of the pharmaceutical composition Gymnemic Acid derivative of formula I and II can be in the form of single dose and taken 1-4 times per day.

According to this invention, the pharmaceutical base or derivative said of formula I Gymnemic Acid derivative can be prepared as follows:

a) crushing dry leaves of Gymnema cane, then extracting three times with 60-95% ethanol under reflux, two hours for each, combining the extracted liquid and concentrating under reduced pressure until there was no ethanol for use;

b) extracting the concentrated mixtures in step a) for 3 to 6 times with cyclohexane, then extracting with n-butanol, concentrating to dryness under reduced pressure, and obtaining dry extract, ready for use;

c) subjecting the dry extracts in step b) to silica gel column chromatography

with elute as mixture of chloroform and methanol at the ratio 90:10 to 60:40, and obtaining derivatives of formula I,

d) If desired, converting the derivative of formula I in step c) into a pharmaceutical base salt thereof.

According to this invention, the Gymnemic Acid derivative of formula II can be prepared as follows:

a) Crushing dry leaves of Gymnema cane, then extracting three times with 60-95% ethanol under reflux, two hours for each, combining extracted liquid and concentrating under reduced pressure until there was no ethanol, ready for use.

b) extracting concentrated mixtures for 3 to 6 times with cyclohexane, then extracting with n-butanol, concentrating to dryness under reduced pressure, ready for use;

c) mixing the dry extracts in step b) with raw silica gel; subjecting separation with thin layer chromatography of silica gel H; the mixture of chloroform and methanol at the ratio 90:10 to 50:50 as elute, subjecting the residue after elute to C_{18} column chromatography with elute as methol/water (20:80-40:60), and obtaining derivative of formula II;

d) if desired, converting the derivative of formula II in step c) into pharmaceutical base salt therof.

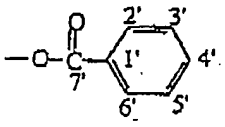
According to this invention, the extract products with 12.5-40 wt% Gymnemic Acid derivative of formula I and formula II can be prepared as follows: raw powder of Gymnema cane leaves were refluxed 1-4 times with 60-95% ethanol, the amount of solvent for each is 6ml/g, extract time is 1-3 hours. The extract mixtures were combined together and distilled under reduced pressure till there was no ethanol, the concentrated mixture was extracted with cyclohexane for 1-3 times, 500ml solvents was used for each time. Then the mixture was extracted for 1-3 times 500ml with n-butanol, all the extract mixtures were combined and distilled under reduced pressure to

obtain the desired product.

This invention gives a further illustration by the preparation examples and biological activity experiment, but it does not means any limitation to the invention.

Example 1

Preparation of compound A (Gymnemic Acid derivative of formula I wherein the R_1 being H) and compound B (Gymnemic Acid derivative of formula I wherein the R_1 being group as follow)



1000g raw powder of Gymnema cane leaves were refluxed for 3 times with 60% ethanol, 6L solvents were used for each, and 2 hours for each time. The extract mixtures were combined together and distilled under reduced pressure till there was no ethanol, the concentrated mixture was extracted with 0.5L cyclohexane and butane for 3 times. All the n-butane extract mixtures were combined and distilled under reduced pressure to obtain 64.0g dry extract product. 32.0g dry extract substance was added into 60g 60-100 mesh rough silica gel, the mixture was vaporized to dryness on a water pan. 450g 200-300 mesh (m) silica gel were loaded into column by a wet method, then treated sample was added to be subjected to column separation with elute as 90:10-60:40 of mixtures of chloroform and methanol, 80mg compound A and 60mg compound B were obtained.

The physics and chemistry data of compound A and compound B were showed as follows:

Compound A:

Amorphous powder: mp198 - 202℃; $[\alpha]_{20}^{D}$+16.0° (c0.10, MeOH); IRν_{max}3414 (OH), 1724 (COOH), 1636 (C=C), 1458, 1380, 1054cm^{-1}; ^{1}HNMR (500MHz, pyridin - d_5) δ0.86 (3H, s, Me), 0.95 (3H, s, Me), 1.01 (9H, s, 3 ×Me), 1.32 (3H, s, Me), 1.39 (3H, s, Me), 3.39(1H, dd, J=4.3 and 11.8Hz, H - 3α), 3.68(1H, d, J=10.5Hz, H - 28a), 4.43 (1H, d, J=10.5Hz, H - 28b), 4.68 (1H, m, H - 16α), 5.04 (1H, d, J=7.8Hz, H - 1 of gluconic acid), 5.26 (1H, brs, H - 12); ^{13}CNMR (125MHz, pyridin - d_5), See table 1 and 2; FAB MSm/z 657[M+Na]$^{+}$.

Compound B:

Amorphous; mp192 - 195℃; $[\alpha]_{20}^{D}$+27.2° (c 0.15, MeOH); IRν_{max}3444 (OH), 1724, 1700, 1635 (C=C), 1457, 1388, 1280, 1074, 720cm^{-1}; ^{1}HNMR (500MHz, pyridin) δ0.98 (3H, s, Me), 1.01 (3H, s, Me), 1.02 (9H, s, 3 ×Me), 1.07 (3H, s, Me), 1.30 (3H, s, Me), 1.34 (3H, s, Me), 1.36 (3H, s, Me), 3.40 (1H, dd, J=4.5 and 12.0Hz, H - 3α), 3.70 (1H, d, J=10.2Hz, H - 28a), 4.42 (1H, d, J=10.2Hz, H - 28b), 4.70(1H, m, H - 16α), 5.10(1H, d, J=7.8Hz, H - 1 of gluconic acid), 5.70 (1H, dd, J=4.7 and 12.3Hz, H - 21α), 7.47 (3H, overlap, H - 3′, - 4′ and - 5′), 8.25 (2H, dd, J=1.4 and 4.8Hz, H - 2′ and - 6′); ^{13}CNMR (125MHz, pyridin - d_5), See table 1 and 2; FAB MSm/z 777[M+Na]$^{+}$.

Table1: ^{13}CNMR data of glucoside liquid of compound A and B

Carbon atom	Compound A	Compound B
1	38.8	38.8
2	26.6	26.6
3	89.0	89.0
4	39.5	39.6
5	55.7	55.7
6	18.4	18.4
7	32.9	33.0
8	40.1	40.1
9	47.1	47.1
10	36.7	36.7
11	23.8	23.9
12	122.6	123.1
13	143.9	142.6
14	43.8	43.7
15	36.7	36.8
16	66.6	66.4
17	41.1	43.8
18	44.4	44.2
19	47.1	47.2
20	31.1	36.0
21	34.3	75.6
22	26.2	33.3
23	28.2	28.2
24	16.9	16.9
25	15.7	15.7
26	17.0	17.0
27	27.2	27.0
28	68.9	66.8
29	33.4	29.2
30	24.1	18.8
Acyl 1′		131.6
Acyl 2′		129.9
Acyl 3′		128.9
Acyl 4′		133.2
Acyl 5′		128.9
Acyl 6′		129.9
Acyl 7′		166.3

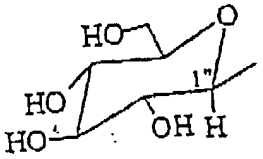
Table 2: ^{13}CNMR data of saccharide part compound A and B

Carbon atom of C-3	Compound A	Compound B
Glutamic acid 1	107.3	107.3
Glutamic acid 2	75.6	75.6
Glutamic acid 3	78.2	78.2
Glutamic acid 4	73.5	73.6
Glutamic acid 5	77.8	77.7
Glutamic acid 6	173.1	173.3

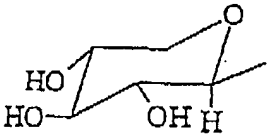
3-position substitution

Example 2:

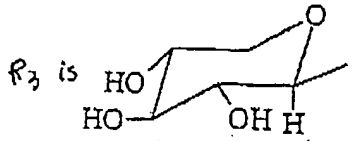
Preparation of Compound C (formula II Gymnemic Acid derivative with R_3 as H and R_2 as follow group),



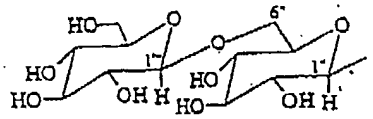
compound D (formula II Gymnemic Acid derivative with R_3 as follows and R_2 as H),



compound E(formula II Gymnemic Acid derivative with R_3 as follow),



R_2 as follow and compound F(formula II Gymnemic Acid derivative with R_3 as H and R_2 as follow)



1000g raw powder of Gymnema cane leaves were refluxed for 3 times with 75% ethanol. 6.0L solvents were used, 2 hours for each time. The extract mixtures were combined together and distilled under reduced pressure till there was no ethanol, the condensed mixture was extracted with 0.5L cyclohexane and butane for 3 times. All the n-butane extract mixtures were gathered and distilled under reduced pressure to obtain 72.0g dry extract product. 36.0g dry extract substance was taken and added into 60g 60-100 mesh rough silica gel, the mixture was vaporized to dryness on a water pan

400g 200-400 mu silica gel H used as thin-layer separation were loaded into column in a wet method, then treated sample was added undergoing column separation with elute as 90:10-50:50 chloroform-methanol mixtures, 130mg compound C, 115mg compound D , 160mg compound E and 195mg compound F were obtained respectively.

The physics and chemistry data of compound C were shown as follows:

Amorphous powder; mp206 - 209℃; $[\alpha]_{20}^{D}$ - 16.0° (c 0.11, MeOH); IRν_{max}3424 (OH), 1735 (COOR), 1636 (C=C), 1457, 1034cm^{-1}; ^{1}HNMR (400MHz, pyridin - d_5) δ0.82 (3H, s, Me), 0.87 (3H, s, Me), 0.91 (3H, s, Me), 0.97 (3H, s, Me), 1.07 (3H, s, Me), 1.20 (3H, s, Me), 1.23 (3H, s, Me), 3.17 (1H, dd, J=3.5 and10.2Hz, H - 18), 3.30 (1H, d, J=3.9 and 11.7Hz, H - 3α), 5.37 (1H, brs, H - 12), ^{13}CNMR (100MHz, pyridin - d_5), See table 3 and 4; FAB MSm/z 943[M+H]$^{+}$.

The physics and chemistry data of compound D were shown as follows:

Amorphous powder; mp202 - 204℃; $[\alpha]_{20}^{D}$ - 3.2° (c 0.15, MeOH); IRν_{max}3410 (OH), 1710 (COOR), 1638 (C=C), 1458, 1036cm^{-1}; ^{1}HNMR (400MHz, pyridin - d_5) δ0.87 (3H, s, Me), 0.91 (3H, s, Me), 0.96 (3H, s, Me), 1.02 (3H, s, Me), 1.10 (3H, s, Me), 1.24 (3H, s, Me), 1.29 (3H, s, Me), 3.30 (1H, dd, J=4.5 and 11.5Hz, H - 3α), 5.38 (1H, brs, H - 12), ^{13}CNMR (100MHz, pyridin - d_5), See table 3 and 4; FAB MSm/z 935[M+Na]$^{+}$.

The physics and chemistry data of compound E were shown as follows:

Amorphous powder; mp212 - 215℃; $[\alpha]_{20}^{D}$ - 9.6° (c 0.20, MeOH); IRν_{max}3414 (OH), 1740 (COOR), 1636 (C=C), 1460, 1364, 1044, 896cm^{-1}; ^{1}HNMR (500MHz, pyridin - d_5) δ0.85 (3H, s, Me), 0.90 (3H, s, Me), 0.94 (3H, s, Me), 1.00 (3H, s, Me), 3.19 (1H, dd, J=4.0 and 13.7Hz, H - 18), 3.32 (1H, d, J=4.4 and 11.7Hz, H - 3α), 5.40 (1H, brs, H - 12), ^{13}CNMR (100MHz, pyridin - d_5), See table 3 and 4; FAB MSm/z 943[M+Na]$^{+}$.

The physical and chemical data of compound F were shown as follows:

Amorphous powder; mp209 - 211℃; $[\alpha]_{20}^{D}$ - 12.1° (c 0.12, MeOH); IRν_{max}3424 (OH), 1734 (COOR), 1636 (C=C), 1458, 1047cm^{-1}; ^{1}HNMR (400MHz, pyridin - d_5) δ0.87 (3H, s, Me), 0.90 (3H, s, Me), 0.92 (3H, s, Me), 1.00 (3H, s, Me), 1.09 (3H, s, Me), 1.22 (3H, s, Me), 1.26 (3H, s, Me), 3.20 (1H, dd, J=3.5 and 13.6Hz, H - 18), 3.33 (1H, d, J=4.4 and 11.5Hz, H - 3α), 5.39 (1H, brs, H - 12), ^{13}CNMR (100MHz, pyridin - d_5), See table 3 and 4; FAB MSm/z 1127[M+H]$^{+}$.

Table3: ^{13}CNMR data of glucoside ligand of compound C-F

Carbon atom	Compound C	Compound D	Compound E	Compound F
1	38.8	38.7	38.7	38.7
2	26.6	26.7	26.7	26.7
3	88.9	89.0	89.0	89.0
4	39.4	39.5	39.5	39.5
5	55.7	55.8	55.8	55.8
6	18.4	18.3	18.5	18.5
7	33.0	33.1	33.1	33.1
8	39.8	39.9	39.9	39.9
9	47.9	48.0	48.0	48.0
10	36.9	37.0	37.0	37.0
11	23.7	23.7	23.8	23.7
12	122.9	122.8	123.0	122.9
13	144.0	144.4	144.0	144.1
14	42.0	42.1	42.1	42.1
15	28.2	28.2	28.2	28.2
16	23.3	23.4	23.4	23.4
17	46.9	46.5	47.0	47.0
18	41.6	41.9	41.7	41.7
19	46.2	46.1	46.2	46.3
20	30.7	30.9	30.8	30.8
21	33.9	34.4	34.0	34.0
22	32.5	33.1	32.5	32.5
23	28.1	28.2	28.2	28.3
24	17.0	17.0	17.0	17.0
25	15.5	15.8	15.6	15.6
26	17.4	17.3	17.5	17.5
27	26.0	26.1	26.1	26.1
28	176.4	180.2	176.5	176.5
29	33.1	33.2	33.2	33.2
30	23.6	23.7	23.7	23.7

Table 4: ^{13}CNMR data of saccharic part of compound C-F

~~C-3~~	Compound C	Compound D	Compound E	Compound F
Glc1	106.9	107.0	107.0	106.9
Glc2	75.1	75.0	75.0	75.2
Glc3	78.4	78.3	78.3	78.4
Glc4	71.6	71.5	71.5	71.5
Glc5	77.0	77.0	77.0	77.0
Glc6	70.4	70.4	70.4	70.5
Glc'1	105.4	105.4	105.4	105.4
Glc'2	75.5	75.6	75.6	75.6
Glc'3	78.5	78.5	78.5	78.6
Glc'4	71.7	71.6	71.6	71.7
Glc'5	78.4	76.9	76.9	78.5
Glc'6	62.7	69.8	69.8	62.6
Xyl1		106.0	106.0	
Xyl2		74.9	74.9	
Xyl3		78.0	78.1	
Xyl4		71.1	71.1	
Xyl5		67.0	67.1	
~~C-28~~				
Glc''1	95.7		95.8	95.7
Glc''2	74.1		74.1	73.9
Glc''3	78.8		78.9	78.7
Glc''4	71.0		71.1	70.9
Glc''5	79.3		79.3	78.0
Glc''6	62.1		62.2	69.3
Glc'''1				105.3
Glc'''2				75.2
Glc'''3				78.5
Glc'''4				71.7
Glc'''5				78.4
Glc'''6				62.7

Biological activity experiments

Experiment example 1

Effect of compound B on the increasing blood sugar in rats caused by sucrose.

Female SD rats fasted for 24 hours were divided into several groups randomly. Test groups are given 50,100,200mg/kg compound B, the positive-control group was given 100mg/kg phenformin, The normal group, control group and blank group were given the same amount of water, the given medicine volume was 10mg/kg, after 30 minutes each group was given saccharose 1/kg(5ml/kg) except normal group, and blood was extracted from the eyes of rats after 30, 60 and 120 minutes respectively, the content of glucose in the serum was measured.

The result was, after the rats were given saccharose for 30, 60 minutes, the value of blood sugar increased apparently. The compound B 200mg/kg and phenformin 100mg/kg within 30 minutes can both reduce the increased value of blood sugar remarkably, and the strength of the two compounds was similar. Results see table 5.

Table 5: The effect of compound B on the increasing blood sugar in rats caused by sucrose.

(X±SD, n=10)

group	dose (mg/kg)	Value of blood sugar (mmol/L)		
		30minutes	60 minutes	120 minutes
Normal group		3.56±0.64	4.12±0.72	3.76±0.69
control group		6.58±0.87$^{\Delta\Delta}$	5.93±1.27$^{\Delta\Delta}$	4.54±1.37
compound B	50	6.03±0.86	6.42±0.78	4.26±1.03
	100	5.12±1.29**	5.77±1.09	4.53±0.94
	200	4.43±0.72**	4.73±0.83**	4.07±0.70
phenformin	100	4.24±0.87**	4.74±0.90*	4.79±1.03

$^{\Delta\Delta}$P<0.01, compared with normal group; *P<0.05, *P<0.01, compared with control group.

Experiment example 2

The effect of compound B on the contents of TG, cholesterol in serum of hyperlipidemia rats

Male SD rats with the weight of 130-170g, normal group was given common food, other groups were given food having high lipid content (1%cholesterol, 10%lard, 0.3% cholic acid, 0.2% methylthio imidazole and 88.5% common forage, made into block.). In sequential ~~14~~ days, rats fasted 12 hours were measured by reagent box method to obtain the contents of TG and cholesterol in serum. Then they were divided according to the value of blood ~~grease~~ into different group. The experimental group was given 50, 100. 200mg/kg compound B, the positive-control group was given clofibrate 100mg/kg, the confro group was given water, the given medicine volume was 10ml/kg, for 10 days, each group was still given high fat forage for 5 days before given medicine, common forage was given in the later 5 days, the rats were fasted for 11 hours before being given the final administration and blood of rat were extracted to obtain the content of TG and cholesterol in serum 1 hour after being given medicine.

The results show that 10 days after rats were given forage having high grease, the contents of TG and cholesterol increased apparently, compound B 50, 100, 200mg/kg and clofibrate 100mg/kg can both reduced the contents of TG and cholesterol in blood serum of hyperlipidemia rats, compound B 200mg/kg ~~have~~ has the same effect as 100mg/kg clofibrate in reducing hyperlipidemia, see table 6

Table 6: The effect of compound B on the contents of blood lipid in hyperlipidemia rats

(X±SD, n=9 - 10)

group	dose (mg/kg)	TG (mmol/L)		Total cholesterol (mmol/L)	
		Before administration	After administration	Before administration	After administration
Normal group		1.02±0.22	1.04±0.15	2.43±0.41	1.99±0.47
control group		2.64±0.82	3.04±0.93	4.10±0.51$^{\Delta\Delta}$	4.77±0.63$^{\Delta\Delta}$
compound B	50	2.72±0.61	2.41±0.44	4.29±0.60	3.92±0.58**
	100	2.54±0.90	1.75±0.53**	4.02±0.59	2.94±0.66**
	200	2.72±0.76	1.37±0.40**	4.18±0.61	2.31±0.74**
clofibrate	100	2.51±0.77	2.72±0.74	4.33±0.51	2.15±0.76**

$^{\Delta\Delta}$P<0.01, compared with normal group; **P<0.01, compared with control group

Experiment example 3

Effect of compound B on blood platelet aggregation in rabbits invitro.

Taken blood from rabbit heart with puncture, 3.8 % potassium citrate for articoagulation (1:9), centrifuge 15 minute in 1000rpm, take upper layer as rich blood platelet plasma (prp), And then, centrifuge 10 minutes with 4000rpm, take supernatant as poor blood platelet plasma (ppp). Transfer PPP(200ul) to nephelotube, and add into different concentration physiological brine solution 10ul of the compound B, final concentration is respectively 250,500,1000μg/ml, add physiological brine 10μl of aspirin to positiye control tube, ~~put~~ it into measuring cell after warming for 2 minutes in 37℃, ~~and add~~ into physiological brine solution10ul of ADP sodium salt with stirring, final concentration is 1.0×10^{-5}M. Observe the maximal aggregation ratio on PAM-1 type of blood platelet instrument within 3 minutes.

The result shows that the compound B 500,1000ug/ml and aspirin 250ug/ml obviously inhibit blood platelet from aggregating.

Table 7: Effect of compound B on blood platelet aggregation in rabbit (X± SD, n=8)

Group	Final concentration	Maximal aggregation ratio (%)	Inhibition ratio
control group		47.9±5.2	
compound B	250	43.6±7.0	9.0
	500	35.9±4.5**	25.1
	1000	27.8±4.8**	42.0
Aspirin	250	23.7±6.0**	50.3

**P<0.01,compared with control group

Experiment example 4

Effect of compound F on blood sugar elevation in rat.

Male Kun Ming strain mice are divided into randomly experimental groups, they respectively take orally the compound F 50,100,2000mg/kg, the positive control group respectively take orally glybenclamide 50mg/kg, blank control group and normal control group take orally the same distilled water, the volume of medicine given is 20ml/kg, lasting 7 days. They are forbidden to give feedstuff 10 hours before the last time of administration. Each group is given dextrose solution 2.5g/kg (10ml/kg) except of normal control group. Before and after 30 minute of administration of dextrose, pick blood 100ul from eyepit, measure the content of dextrose in serum according to the way of dextrose oxygenation enzyme.

Result, after mice take orally dextrose 30 minutes, blood sugar obviously rise. Both the compound F 100,200mg/kg and 50mg/kg obviously inhibit blood sugar in mice from rising. The function of the compound B 200mg/kg and glybenclamide 500mg/kg lowering blood sugar is similar, which may be seen in table 8.

Table 8

Group		Dose (mg/kg)	Value of blood sugar
		0 minute	30 minutes
Normal group		6.20±1.01	6.64±1.04
control Group		6.55±1.16	13.94±3.22
compound (B) F	50	6.79±1.16	12.01±1.88
	100	6.09±1.34	9.59±2.25**
	200	6.42±0.99	9.16±1.08**
glybenclamide	50	4.48±0.83**	8.18±1.72**

P<0.01,compared with normal group: **p<0.01,compared with control group

Experiment example 5

Effect of compound F on the content of triglycerides and cholesterol in the serum of hyperlipidemia rat.

Male SD rat with weight of 130-170g. The normal group is given general feedstuff, and the other groups are given high-fat (1% cholesterol, 10% pig oil, 0.3% clolic acid ,0.2 % methylthio imidazole and 88.5 % normal feedstuff are made stuff by oneself).After the feedstuff is run for 14 days and the mice rat is forbidden to eat for 12 hours, measure the content of triglycerides and cholesterol in rat's serum. And then, the rat are grouped randomly according to blood lipid value .The experiment group is given to this compound F (50,100,200mg/kg), the positive-control group is given orally to clofibrate of (100mg/kg) the control group is given distilled water. The volumn of administration is given 10ml/kg, lasting 10 days. Each group is given

high-fat feedstuff in the former 5days of giving drugs, and then general feedstuff in the next 5days.Fasting of 11 hours is conducted before the last time of giving drugs. After giving drugs for 1 hour, take blood and measure the content of ester and cholesterol in the blood serum.

Result

The content of TG and cholesterol in the blood serum of rat elevates obviously after given high-fat feedstuff for 10 days.50mg/kg, 100mg/kg, 200mg/kg of compound F and 200mg/kg clofibrate make the level of triglycerides and cholesterol in blood serum of rat with high-fat blood diseases lower. The action 200mg/kg of the compound F is the similar as to that of 100mg/kg of clofibrate in the function of lowering blood fat.(table 9)

Table 9:the effect of compound F on the content of blood fat of rat with high-fat blood disease. (X±SD, n=9—10)

group	dose (mg/kg)	triglycerides (mmol/L)		Total cholesterin (mmol/L)	
		Before administration	After administration	Before administration	After administration
Normal group		1.02±0.22	1.04±0.15	2.43±0.41	1.99±0.47
Control group		2.64±0.82	3.04±0.93	4.10±0.51$^{\Delta\Delta}$	4.77±0.63$^{\Delta\Delta}$
compound F	50	2.72±0.61	2.41±0.44	4.29±0.60	3.92±0.58**
	100	2.54±0.90	1.75±0.53**	4.02±0.59	2.94±0.66**
	200	2.72±0.76	1.37±0.40**	4.18±0.61	2.31±0.74**
clofibrate	100	2.51±0.77	2.72±0.74	4.33±0.51	2.15±0.76**

$^{\Delta\Delta}$P<0.01, (compared with normalgroup); **P<0.01(compared with control group)

Experimental example 6, effect of compound F affects on blood platelet aggregation in rabbit.

Take blood from rabbit heart by puncturing,3.8% of potassium citrate for

anticoagulation (1:9), centrifuging 15 minute in 1000rpm, take the upper layer as blood platelet rich plasma(prp), And then centrifuga 10 minute in 4000rpm, take supernatant as blood platelet poor plasma(ppp). the final concentration of compound B is revetively 250,500,1000 μ g/ml, final concentration is respectively250,500,1000ug/ml, add 10ul of physical brine of aspirin to the positive-control tube to final concentration of 250 μ g/ml, add 10ul of physical brine to control tube to the final concentration of 250 μ g/ml .Observe the maximal aggregation ratio on PAM-1type instrument of blood platelet aggregation within 3 minute.

The result shows that 500,1000μg/ml of the compound F and aspirin 250μg/ml obviously inhibit the aggregation of blood platelet.

Table 10: the effect of the compound F on aggregation of rabbit's blood platelets in vitro.

group	Final concontration (μg/ml)	Maximal aggregation rate (%)	Inhibition rate (%)
control		47.9±5.2	
compound F	250	43.6±7.0	9.0
	500	35.9±4.5**	25.1
	1000	27.8±4.8**	42.0
aspirin	250	23.7±6.0**	50.3

**P<0.01(compared with the control)

Experimental example 7

Effect of compound B on blood sugar in normal mice.

Male Kun Ming strain mice are divided into randomly experimental groups, they respectively take orally the compound B 50,100,2000mg/kg, positive control group respectively take orally tolbutol 100mg/kg, blank control group takes orally the same distilled water, the volume of medicine given is 20ml/kg, lasting 14 days. They are administrated to test drug (provided that they are pre-forbidden to give food 5 hrs before administration) after the days 1, 3, 7, 14 of administration. After 3hrs of administration, pick

blood (10ul) from eyepit, measure the content of dextrose in serum according to the way of reagent box.

Result shows that compound B 50, 100, 200mg/kg for continucly administration 14 days has no obvious effect on blood sgar of normal onice, but tolbutol starting from day 3 of administration show obvious effect for lowering the blood sugar of normal mice. Result is also seen in table 11.

Table 11, Effect of compound B on blood sugar in normal mice.

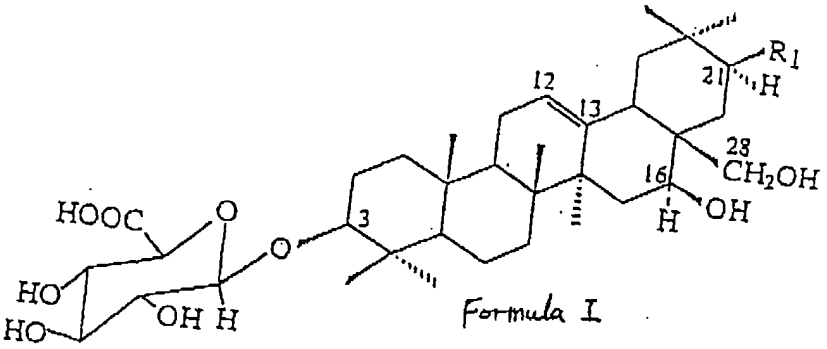
(X±SD, n=10)

Group	Dosc (mg/kg)	Value of blood sugar			
		1	3	7	14 (day)
control Group		5.21±1.10	7.10±1.30	8.56±0.74	7.52±1.29
compound B	50	5.84±0.94	7.56±0.92	8.51±1.06	8.27±0.66
	100	6.48±1.28	7.73±2.26	8.71±0.97	7.45±1.59
	200	6.41±1.04	6.28±1.19	8.46±0.88	7.86±1.56
tolbutol	100	6.48±1.18	5.22±0.80**	6.62±0.96	5.75±1.02

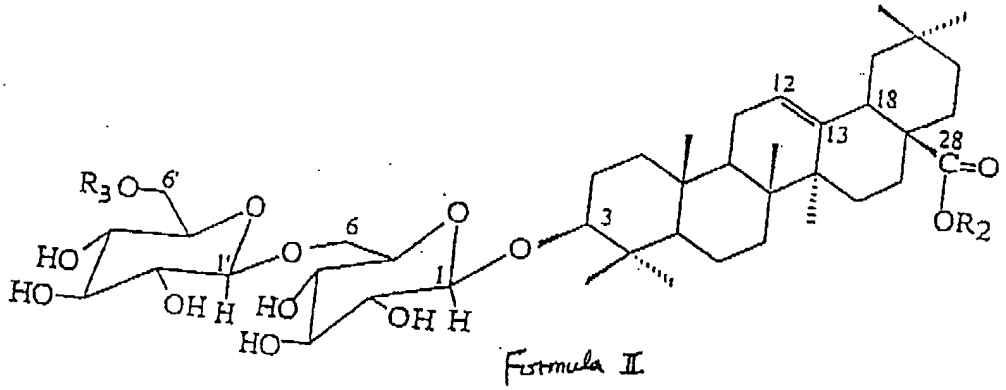
P<0.01,compared with normal group: **p<0.01,compared with control group

What is claimed

1. Gymnemic Acid derivative of general formula I or general formula II,

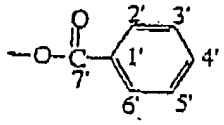


Formula I

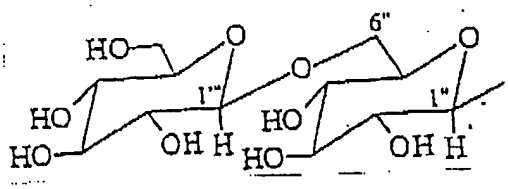


Formula II

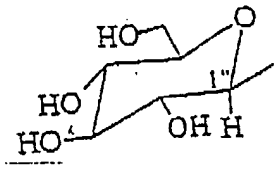
wherein, R_1 is H or the radical represented by the following formula



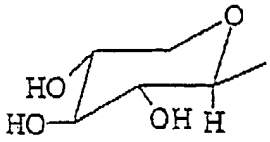
R_3 is H, and R_2 symbolizes the following radical, or



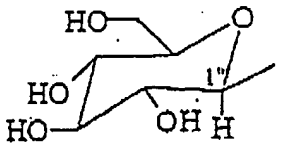
or



R_3 symbolizes the following radical,



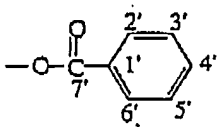
R_2 is H or the following radical ,



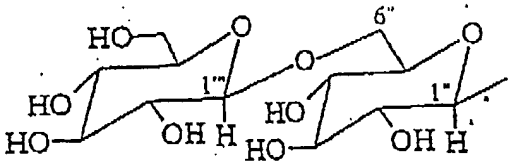
or pharmaceutically base addition salt thereof.

2.Gymnemic Acid derivatives of claim 1,wherein R_1 in formula I is hydrogen.

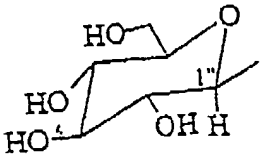
3.Gymnemic Acid derivatives of claim 1,wherein R_1 in formula I is a group of the formula:



4.Gymnemic Acid derivatives of claim 1, wherein R_3 in formula II is hydrogen, R_2 is group of formula:

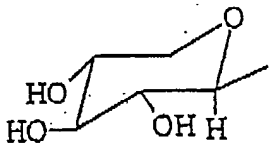


5. Gymnemic Acid derivatives of claim 1, wherein R_3 in formula II is hydrogen, R_2 is group of formula:

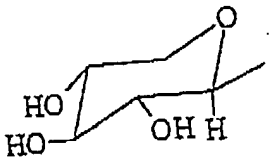


6. Gymnemic Acid derivatives of claim 1, wherein R_2 in formula II is hydrogen,

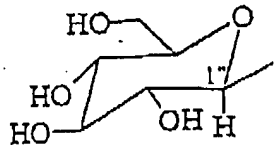
R_3 is group of formula:



7. Gymnemic Acid derivatives of claim 1, wherein R_3 in formula II is group of formula



R_2 is group of formula:



8. Pharmaceutical composition which contains at least one kind of Gymnemic Acid derivative of formula I and/or II or pharmaceutical base addition salt thereof as active ingredient, pharmaceutical carrier and excipient.
9. Pharmaceutical composition for the prevention or treatment of the diseases associated with hyperglycemia, hyperlipidemia and platelets aggregation, which contains at least one kind of Gymnemic Acid derivative of formula I and/or II or pharmaceutical base addition salt thereof as a active ingredient, pharmaceutical carrier and excipient.
10. A composition of claims 1 or 2,which contains Gymnemic Acid derivative of formula I and/or II ,wherein based on the weighe of the composition,the content of compounds A,B,C,D,E and is 1.25-2.10% compound A,

0.89-1.50% compound B, 2.40-3.80% compound C, 2.10-3.40% compound D, 2.74-4.60% compound E and 3.24-5.40% compound F.

11. A extract of Gymnema sylevestre.R.Br which contains 12.5-40wt% Gymnemic acid derivatives of formula I and formula II.

12. Use of Gymnemic Acid derivative of formula I and II or the extract containing Gymnemic Acid derivative of formula I and II for the manufacture of medicament for the prevention or treatment of the diseases and conditions associated with hyperglycemia, hyperlipidemia and platelets aggregation.

13. A method of the preparation of Gymnemic Acid derivative of formula I and II or pharmaceutical base addition salt thereof, which includes the following steps:

c) extracting the plant Gymnema cane with ethanol under reflux and then concentrating;

d) extracting concentrated liquid in step a) with cyclohexane , then extracting with n-butanol, concentrating to dryness under reduced pressure, and then obtaining a ointment;

c) subjecting the ointment in step b) to silica column chromatography with elute as chloroform: methanol=90:10—50:5 or 90:10—60:40, obtaining Gymnemic acid derivative of formula I and residue;

d) subjecting the residue in step c) to C_{18} column chromatography with elute as methal/water (20/80-40/60), obtaining Gymnemic acid derivative of formula II;

e) if desired, converting the obtained Gymnemic acid derivative of formula I or II into pharmaceutical base addition salt with inorganic or organic base.

Summary/Abstract

This invention relates to new Gymnemic acid derivatives, their preparation, pharmaceutical composition or extract which contains them, and their medical use, especially the use in the prevention or treatment of the diseases associated with hyperglycemia, hyperlipidemia and platelets aggregation.